1981

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Environmental Health Perspectives
Vol. 40, pp. 267-278, 1981

Use of *Lilium longiflorum*, cv. Ace Pollen Germination and Tube Elongation As a Bioassay For the Hepatocarcinogens, Aflatoxins

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Although various animal tissues are used for bioassay of aflatoxins (B_1, B_2, G_1, G_2), a rapid bioassay dependent upon a plant part's response does not exist. Both pollen germination (G) and tube elongation (TE) were enhanced in a 3.0 *mM* KH_2PO_4 (K)-containing but AFB_1-lacking, modified Dickinson's medium. The B_1 did not affect G when K was withheld but K supplementation impaired G above 15 μg/ml B_1. Without K, 5-20 stimulated but 25 and 30 μg/ml B_1 inhibited TE which was suppressed by every B_1 conc tested in K-containing medium. Addition of NaH_2PO_4(N) instead of K to medium did not promote G. Slight G stimulation occurred at 16.6μg/ml mixed aflatoxins (MA) in medium lacking either K or N but low G inhibitions were observed with K or N. The MA at 33.3 μg/ml reduced G 2.5% in K's or N's absence and 26 or 17% in their presence. While K did not stimulate TE without MA, N did 26%. At 16.6 and 33.3 μg/ml MA, TE was reduced 19, 6, 19% and 24, 25, 31%, respectively, in control, K- and N- media. Pollen G and TE were markedly sensitive to G_1. Significant inhibitions of *Zea mays* seed G were observed at 5.8 and 11.6 μg/ml B_1 but not root elongation (RE) from 0.4-11.6 μg/ml. The MA (31.5 μg/ml) administered for 72-240 hr did not influence either *Arachis hypogeae* seed G or RE. However, imbibing 5 cultivars each of *Avena sativa* (65-117 hr) and *Hordeum vulgare* (39-89 hr) inhibited RE 4/15-62%. Thus, except for *Z. mays*, pollen G and TE appear to be more B_1-sensitive than seed G and RE. But, the pollen bioassay is less sensitive than both certain animal bioassays (0.025 μg/ml) and analytical methodologies (10 pg.)

Introduction

Although a number of bioassays for aflatoxin B_1 (AFB_1) and certain other aflatoxins exist which employ a variety of animals (*1*), a bioassay which utilizes a plant or alternatively one or more of its parts has not been developed. However, there are published reports, which have been reviewed by Dashek and Llewellyn (*2*), that describe the effects of aflatoxins on seed germination, seedling growth or elongation, chlorophyll synthesis, enzymatic activities, amino acid uptake, protein or nucleic acid syntheses and cellular ultrastructure of or by a variety of plant parts. A number of these effects could possibly be of service in the development of a bioassay.

Because our initial investigations (*3*) suggested that both *Lilium longiflorum*, cv. Ace pollen germination and tube elongation responded to AFB_1 at concentrations as low as 4 μg/ml (approximately 30% inhibition of germination) and 8 μg/ml (about

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This report is part of a series of papers on Pollen previously published in Environ. Health Perspect. 33: 1-165 (1980).

25% inhibition of tube elongation), we have attempted to develop a bioassay utilizing these two parameters of pollen growth and development. This paper reports the results of that attempt, an examination of aflatoxin effects on seed germination and elongation of attached roots of certain crop plants, a comparison of the sensitivities of various plant and animal tissues to aflatoxins and a comparison of the sensitivities of the pollen bioassay and analytical methods for quantitating aflatoxins.

Materials and Methods

Pollen Germination Conditions

Experiments with AFB_1 and KH_2PO_4. Two stock solutions of Dickinson's medium (*4*) without tetracycline were adjusted to pH 5.2; the control and experimental stocks lacked and contained 3.0 mM KH_2PO_4, respectively. Aflatoxin B_1 (A grade, Calbiochem., LaJolla, Calif.) was dissolved in 7 ml of acetone which was then added to 500 ml of experimental medium to yield 30 μg/ml toxin. This concentration was verified by combined thin layer chromatography and a visual dilution technique which is sensitive to 2 ppb (*5*). Acetone (7 ml) was supplied to the control medium prior to autoclaving. The experimental medium was diluted to yield media containing 5, 10, 15, 20 and 25 μg/ml toxin.

Lilium longiflorum, cv. Ace pollen (6 months, 4°C) was added in lots of 10 mg fresh weight to 20 ml aliquots of sterile medium in sterile, disposable Petri dishes which were then incubated at 26 ± 2°C for 4 hr. To measure tube lengths and assess percent germination, photomicrographs of the dishes were taken subsequent to positioning the dishes over a partitioned grid. The germination percentages and tube lengths presented in Table 1 are means and standard deviations for three experiments. Each of these experiments had two controls. The total number of grains and tubes which were scored and measured, respectively, was 350 per treatment.

Experiments with Mixed Aflatoxins and Either KH_2PO_4 or NAH_2PO_4. Because the media which contained or lacked 3.0*mM* KH_2PO_4 differed not only in phosphate but also their potassium contents, we examined the possibility that the K cation rather than the PO_4 anion either stimulated or inhibited pollen germination and/or tube elongation. Therefore, pollen was germinated in medium with or without 3.0*mM* KH_2PO_4 or NaH_2PO_4. Mixed aflatoxins (5 μg/ml AFB_1, 0.2 μg/ml AFB_2, 27.5 μg/ml AFG_1 and 0.5 μg/ml AFG_2) in chloroform were added to Petri dishes at 16.6 and 33.3 μg/ml. Following evaporation of the chloroform, 20 ml of sterile medium and 20 mg fresh weight lots of pollen were added to the Petri dishes which were incubated at 26 ± 2°C for 4 hr. Then, 1 ml aliquots of a 40% formaldehyde solution were pipetted into the media. Pollen tube lengths within media drops, which were removed at random, were measured with a microscope equipped with an ocular micrometer. The experiment was repeated twice and within each experiment there was a replicate for each treatment. Although the total number of pollen grains scored for percent germination of each

Table 1. Germination and tube elongation for pollen sown in medium containing or lacking AFB_1 and KH_2PO_4.[a]

AFB_1 Concentration, μg/ml	Tube length, μm				Germination, %			
	With KH_2PO_4	% Change	No KH_2PO_4	% Change	With KH_2PO_4	% Change	No KH_2PO_4	% Change
0	812 ± 53	—	757 ± 22	—	44.3 ± 3.8	—	29.5 ± 2.8	—
5	795 ± 14	− 0.9	814 ± 29	+ 7.5	45.9 ± 2.8	+ 3.6	34.0 ± 2.2	+15.3
10	727 ± 37	−10.5	865 ± 17	+14.3	46.4 ± 15.5	+ 4.7	38.7 ± 4.7	+31.2
15	770 ± 47	− 5.2	882 ± 49	+16.5	39.6 ± 7.7	−10.6	32.7 ± 2.9	+ 7.5
20	793 ± 14	− 2.3	857 ± 17	+13.2	41.5 ± 6.1	− 6.3	35.1 ± 4.7	+19.0
25	622 ± 33	−23.6	729 ± 33	− 3.7	32.2 ± 2.6	−27.3	30.9 ± 2.4	+ 4.7
30	519 ± 33	−36.1	673 ± 33	−11.1	20.0 ± 1.3	−45.1	29.7 ± 2.5	+ 0.7

[a]Two stock solutions of Dickinson's (4) medium but without tetracycline were adjusted to pH 5.2; one stock lacked 3.0*mM* KH_2PO_4 (control) and the other (experimental) contained it; AFB_1 (grade B, dried *in situ*, Calbiochem, LaJolla, Ca.) was dissolved in 7 ml acetone and then added to 500 ml of the experimental medium with a resulting AFB_1 concentration of 30 μg/ml; subsequent to autoclaving, AFB_1 concentration was verified by thin layer chromatography coupled with a visual dilution technique which is sensitive to 2 ppb; 7 ml of acetone were also added to the control medium prior to autoclaving; the 30 μg/ml AFB_1 experimental medium was diluted to yield media which contained 5, 10, 15, 20 or 25 μg/ml; 10 mg fresh weight lots of stored (6 months, 4°C) *Lilium longiflorum*, cv. Ace pollen were sown in 10 ml aliquots of sterile medium in sterile, disposable, plastic Petri dishes; pollen was incubated for 4 hr at 27 ± 2°C. To obtain tube lengths and percent germinations, photomicrographs were made of the Petri dishes following their positioning over a 3 mm grid; data tabulated from the photomicrographs are means and standard deviations for three experiments; each experiment had two controls; 350 pollen grains were scored and tubes measured per treatment; % change equals the % change from the control; a variant of this table and figure legend will appear (*10*).

treatment varied, that number approximated 3000. The total number of tubes measured was 260.

Experiments with AFB_2, AFG_1 and AFG_2. The specificity of the pollen bioassay was examined by dissolving aflatoxin B_2 (AFB_2) aflatoxin G, (AFG_1) and aflatoxin G_2 (AFG_2) in acetone with subsequent transfer to Petri dishes of aliquots which yielded 15 and 30 μg/ml upon addition of medium which either contained or lacked 3.0*mM* KH_2PO_4.

Seed Germination Conditions

Fifty *Arachis hypogaea*, cv. Florigiant seeds were treated with Botec fungicide, rinsed with sterile H_2O and then sown in lots of five per Petri dish on three layers of sterile standard laboratory paper toweling. The seeds were arranged such that their embryo ends pointed toward the center of the dish. To each dish 15 ml of sterile H_2O containing 31.5 μg/ml mixed aflatoxins (5.6 μg/ml AFB_1, 0.2 μg/ml AFB_2, 25.0 μg/ml AFG_1 and 0.8 μg/ml AFG_2) were added. The dishes were sealed with parafilm and tilted to an angle which permitted the roots to elongate in a straight fashion. The seeds were incubated in the dark at 24 ± 2°C for 72, 144, 168 and 240 hr when percent germination and root lengths were measured, the latter with a mm rule.

The same experimental design was also used for both *Avena sativa* and *Hordeum vulgare* seeds except that five cultivars of each genera were tested. These cultivars were: Coker, Norline, Moregrain, Windsor and Roanoke for *A. sativa* and Surry, Henry, Volbar, McNair and Barsoy for *H. vulgare*. Other differences in the design included 10 seeds and 10 ml of test solution per dish. The germination times were 65, 89 and 177 hr for *A. sativa* and 29, 63 and 89 hr for *H. vulgare*.

Zea mays seeds were surfaced sterilized with 10% Chlorox for 10 min, at which time they were rinsed with 300 ml distilled H_2O. Seeds in lots of 15 were sown on two layers of sterile filter paper and imbibed as above except that the imbibition medium included 50 μg/ml chloramphenicol and the dishes were not tilted. The experiment was replicated three times with duplicate dishes for each treatment within an experiment.

Statistical Analyses

The data were analyzed by a two-tailed *t*-test for evaluating the difference between population means.

Preparation of Mixed Aflatoxins for Seed Germination Studies

Mixed aflatoxins were prepared according to Llewellyn et al. (*6*) through inoculation of coconuts with *Aspergillus parasiticus*, strain NRRL 2999. The inoculated coconuts were extracted with cloroform and the extract analyzed for aflatoxins by the combined thin layer chromatography and visual dilution technique. The choloroform extract contained 1270 μg mixed aflatoxins (225 μg/ml AFB_1, 6 μg/ml AFB_2, 1000 μg/ml AFG_1 and 39 μg/ml AFG_2). A 50 ml portion of this crude extract was transferred to 1 liter of warm sterile distilled water which was gently heated to drive off the chloroform. This solution contained 31.5 μg/ml mixed aflatoxins in the ratio of individual aflatoxins reported above and was stored in the dark at 8°C.

Results and Discussion

Does AFB_1 Inhibit Pollen Germination and Subsequent Tube Elongation When KH_2PO_4 Is Withheld or Included in the Growth Medium?

Germination was not inhibited when pollen was sown in medium lacking phosphate but containing 5-30 μg/ml AFB_1 (Table 1). In contrast, pollen germination was inhibited 10.6, 6.3, 27.3 and 45.1% upon addition of 15, 20, 25 and 30 μg/ml AFB_1 to an incubation medium containing 3.0 *mM* KH_2PO_4. In the absence of KH_2PO_4, only 25 and 30 μg/ml AFB_1 inhibited tube elongation. However, this elongation was inhibited at every AFB_1 concentration when KH_2PO_4 was added to the germination medium. Maximum inhibition occurred at 25 (24%) and 30 (36%) μg/ml.

Do Mixed Aflatoxins Inhibit Pollen Germination and Tube Elongation When Different Phosphate Salts are Withheld or Added to the Growth Medium?

Both percent germination and tube lengths for pollen sown in medium containing or lacking mixed aflatoxins and either KH_2PO_4 or NaH_2PO_4 are shown in Table 2. Without phosphate, 16.6 μg/ml and 33.3 μg/ml mixed aflatoxins stimulated and reduced percent germination by 7.8 and 34.7%, respectively. When 3.0 *mM* NaH_2PO_4 was included in the medium, the percent germination inhibitions were 17% (16.6 μg/ml) and 16.5% (33.3 μg/ml). In contrast, addition of 3.0 *mM* KH_2PO_4 to the incubation medium yielded germination inhibition percentages of 8.3% and 25.6% at 16.6 and 33.3 μg/ml, respectively. Tube elongations for pollen germinated in the absence of phosphate but presence of 16.6 or 33.3 μg/ml mixed aflatoxins were reduced 18.9% and 23.7%, respectively. When the medium was provided

Table 2. Germination and tube elongation for pollen sown in medium containing or lacking aflatoxins and either KH_2PO_4 or NaH_2PO_4.[a]

Aflatoxin concentration, μg/ml	Tube length, μm			Germination, %		
	With KH_2PO_4	With NaH_2PO_4	No PO_4	With KH_2PO_4	With NaH_2PO_4	No PO_4
0.0	826 ± 198	1081 ± 219	860 ± 265	65.3 ± 20.6	53.8 ± 20.4	53.2 ± 17.4
16.64	773 ± 97	879 ± 147	697 ± 131	59.9 ± 22.6	44.6 ± 14.4	57.4 ± 17.7
33.28	616 ± 110	748 ± 156	656 ± 154	48.6 ± 21.2	44.9 ± 16.9	38.9 ± 26.0

[a]Mixed aflatoxins at 16.64 and 33.28 μg/ml (5 μg/ml AFB_1, 0.2 μg/ml AFB_2, 27.5 μg/ml AFG_1, 0.58 μg/ml AFG_2) dissolved in chloroform were added to sterile Petri dishes; following evaporation of the chloroform, 20 ml Dickinson's medium (*4*) minus tetracycline and 20 mg fresh weight of stored (1 month, 4°C) pollen were added to each dish; pollen was germinated 4 hr at 26 ± 2°C in the dark; 1 ml aliquots of 40% formaldehyde were added to the media at 4 hr; tube lengths within drops selected at random were measured with a microscope equipped with an ocular micrometer; the experiment was replicated three times with a replicate for each of the above treatments within an experiment; the number of tubes measured per treatment was 260 and the number of grains scored for determining % germination was variable per treatment but approximated 3,000; data are means and standard deviations; a variant of this table and its legend will appear (*10*).

with 3.0*mM* NaH_2PO_4, tube elongation was suppressed by 18.7% at 16.6 and 30.8% at 33.3 μg/ml mixed aflatoxins. The percent inhibitions of tube elongation were 6.4% (16.6 μg/ml) and 15.4% (33.3 μg/ml) for pollen germinated in medium containing 3.0*mM* KH_2PO_4.

How Specific Is the Response of Pollen to AFB_1?

A comparison of the effects of AFB_2, AFG_1 and AFG_2 on both germination and tube elongation is shown in Table 3. Whereas sowing pollen in medium lacking 3.0*mM* KH_2PO_4 but containing 15 μg/ml AFB_2 did not significantly inhibit percent germination, 30 μg/ml suppressed germination by 29% and tube elongation by 15% (Table 3). Both of these reductions were statistically significant at 95% confidence level. In contrast, both 15 and 30 μg/ml AFG_1 in medium lacking KH_2PO_4 suppressed germination by 97.5 and 99.7%, respectively, and tube elongation by 100%. When KH_2PO_4 was added to the medium, 15 μg/ml AFG_1 decreased germination and tube elongation by 88 and 55%, respectively. However, 30 μg/ml AFG_1 impaired germination by 99.7% and tube elongation by 100%.

The addition of 15 or 30 μg/ml AFG_2 to media which either contained or lacked 3.0*mM* KH_2PO_4 did not inhibit germination except at 30 μg/ml together with KH_2PO_4, but, tube elongation was suppressed 34 and 26% at 15 and 30 μg/ml, respectively, for medium which was not supplemented with KH_2PO_4. Both suppressions were significant at the 95% confidence level. When the medium was provided with 3.0*mM* KH_2PO_4, a 12% (significant) reduction in tube elongation was observed. Therefore, both lily pollen germination and tube elongation appear to be more sensitive to AFG_1 than AFB_1, AFB_2 or AFG_2. However, germination experiments with AFG_1 which would employ pollen of high viability should be performed to substantiate the results reported here for pollen of low viability. In addition, it is desirable to assess the ability of lily pollen to germinate and elongate tubes at < 15 μg/ml AFG_1.

Do Mixed Aflatoxins Affect Seed Germination and/or Root Elongation of Peanuts, Corn, Barley and Oats?

Inclusion of mixed aflatoxins at 31.5 μg/ml in the imbibition medium was without a significant effect on either germination of *Arachis hypogaea* seeds or elongation of their roots at 72, 144, 168 and 240 hr of imbibition (Table 4).

The lack of an aflatoxin effect on either seed germination or elongation of attached *Arachis hypogaea* roots is somewhat surprising, since an aflatoxin incidence rate of 19% in consumer peanut products has been reported for the United States and Canada during the years 1972–1975 (*7*). This unexpected result is coupled with another. Aflatoxin B_1 inhibits the germination and elongation of both attached and excised *Glycine max*, cv. Essex roots (*8-10*), but field-grown soybeans are relatively resistant to invasion by *Aspergillus flavus* (*11*). Aflatoxin was detected in only 2 of 866 soybean samples analyzed by the Department of Agriculture and at total aflatoxin amounts of 10-11 μg/kg.

When *Zea mays* seeds were imbibed in mixed aflatoxins over the concentration range of 0.36-11.60 μg/ml, the percent inhibitions of seed germination were 9.5, 6.0, 13.1, 22.6 and 25% of 0.36, 1.45, 2.90, 5.80 and 11.60 μg/ml mixed aflatoxins, respectively (Table 5). Only the inhibitions which occurred at 5.8

Table 3. Comparison of the effects of AFB_2, AFG_1 and AFG_2 on *Lilium Longiflorum*, cv., Ace pollen germination and tube elongation.[a]

Aflatoxin type	Aflatoxin concentration, μg/ml	With KH_2PO_4		Without KH_2PO_4	
		Germination, %	Tube length, mm	Germination, %	Tube length, mm
AFB_2	0			30.7 ± 12.0	7.5 ± 2.9
	15			31.2 ± 6.8	7.1 ± 0.3
	30			21.8 ± 8.8	6.4 ± 2.8
AFG_1	0	20.6 ± 10.3	5.9 ± 2.3	8.0 ± 5.1	4.6 ± 2.5
	15	2.5 ± 0.9	2.8 ± 1.6	0.2 ± 0.3	0.0 ± 0.0
	30	0.08 ± 0.7	0.0 ± 0.0	0.03 ± 0.06	0.0 ± 0.0
AFG_2	0	13.5	6.6	12.1 ± 4.9	6.9 ± 3.2
	15	15.1	7.4	19.2 ± 8.4	4.6 ± 2.7
	30	14.6	5.8	6.4 ± 7.5	5.1 ± 2.5

[a]Lots of stored (6 months, 4°C) *Lilium longiflorum*, cv. Ace pollen (20 mg fresh weight) were added to 10 ml Dickinson's medium with or without 3.0*mM* KH_2PO_4 and 15 or 30 μg/ml AFB_2, AFG_1 or AFG_2; pollen was germinated in sterile Petri dishes at 26°C for 4 hr when 1 ml of formaldehyde was added to the dishes; drops were removed at random and % germination and tube lengths measured with an ocular micrometer; data are means and standard deviations of four replicates for the AFB_2 and AFG_1 treatments and six replicates for AFG_2 without phosphate; data for AFG_2 with phosphate are averages for two experiments; the number of grains scored was 2300-2900 and tubes measured were 300 per treatment; statistical analyses indicated that the differences in mean germination percentages and tube lengths between toxin-treated and untreated pollen were significant in every case.

Table 4. Effect of aflatoxin on *Arachis hypogaea* seed germination and root elongation.[a]

Time, hr	Germination, %		Root length, mm	
	No aflatoxin	With aflatoxin	No aflatoxin	With aflatoxin
72	4	6	4.0 ± 0.0	3.3 ± 1.2
144	26	30	13.7 ± 11.0	13.0 ± 7.2
168	28	36	21.6 ± 16.7	17.7 ± 10.1
240	40	48	34.5 ± 15.9	34.1 ± 21.2

[a]One hundred peanut seeds were treated with Botec fungicide; 50 seeds were treated with aflatoxin and 50 seeds with H_2O; seeds were arrayed in a straight line on laboratory paper toweling in sterile Petri dishes so that the embryo ends pointed toward the dish's center; 15 ml of sterile H_2O containing 31.5 μg/ml mixed aflatoxins or 15 ml H_2O were added to each dish; the dishes were sealed with parafilm and tilted to obtain an angle which allowed the roots to elongate linearly in order to facilitate measurement; seeds were germinated for the above times in the dark at 26°C when root lengths were measured with a mm rule; root data are means and standard deviations for seeds in ten dishes (five seeds/dish).

and 11.6 μg/ml were statistically significant. As for the effects of mixed aflatoxins on *Zea mays* root elongation, 0.36, 1.45, 2.90 and 5.80 μg/ml stimulated this elongation by 6.1, 30.6, 38.8 and 12.2%. Only the stimulation at 2.90 was statistically significant. There was no effect of 11.60 μg/ml mixed aflatoxins on root elongation. The observed suppressions of seed germination at both 5.8 and 11.6 μg/ml are disturbing, since there are a number of reports which demonstrate that ears of corn can be contaminated with the toxin (*12-16*). The incidence of aflatoxins in corn and corn meal for Southeastern United States in 1969, 1970 and 1974 was 41% (*7*). In this connection, during a single week in August of 1977, 78% of the preharvest corn samples from a 31 county survey in Georgia contained over 100 ppb total aflatoxins (*16*).

Imbibition of seeds of various *Avena sativa* cultivars in a solution containing 31.5 μg/ml mixed aflatoxins yielded the following root inhibitions: 32.3, 43.3 and 66.7% (cv., Norline), 4.3, 33.1 and 68.6% (cv., Windsor), 20.5, 37.2 and 65.8% (cv., Coker), 23.4, 46.2 and 68.8% (cv., Moregrain) and 30.3, 42.4 and 61.8% (cv., Roanoke) at 65, 89 and

Table 5. Effect of aflatoxin on *Zea mays* germination and root elongation.[a]

Aflatoxin concentration, μg/ml	Germination, %	Root length, mm
0 (control)	84 ± 10.5	4.9 ± 0.40
0.36	76 ± 9.9	5.2 ± 0.40
1.45	79 ± 7.1	6.4 ± 0.53
2.90	73 ± 8.5	6.8 ± 0.60
5.80	65 ± 15.4	5.5 ± 0.50
11.60	63 ± 8.2	4.8 ± 0.42

[a]Seeds were surfaced sterilized in 10% Chlorox for 10 min and then rinsed five times in 300 ml distilled H_2O prior to sowing; seeds in lots of 15 were placed in sterile Petri dishes containing a single layer of filter paper; 10 ml of aflatoxin solution was added to each dish, and the dishes were wrapped and allowed to incubate 40 hr at 24°C; at 40 hr, percent germination and root elongation were determined by using a dissecting microscope; to measure uptake 1 ml germination medium was removed from each dish prior to and following germination; the data are means and standard deviations of three experiments with duplicate dishes for each treatment within an experiment.

117 hr of imbibition, respectively (Table 6).

The effects of 31.5 μg/ml mixed aflatoxins on seed germination and root elongation of various *Hordeum vulgare* cultivars are summarized in Table 7. The percent inhibitions of root elongation were 22.4, 38.0 and 59.7% (cv., Surry), 36.5, 42.6 and 61.4% (cv. Barsoy), 35.2, 47.8 and 58.0% (cv., Volbar), 26.8, 36.7 and 61.2% (cv., McNair) and 15, 36 and 62.2% (cv. Henry) for seeds imbibed 39, 63 and 89 hr, respectively.

As for the incidence of aflatoxins in small grains in "commercial channels" in the United States

Table 6. Effect of aflatoxin on seed germination and root elongation of various cultivars of *Avena sativa*.[a]

Avena sativa cultivar	Time, hr	Germination, %		Root length, mm	
		No aflatoxin	With aflatoxin	No aflatoxin	With aflatoxin
Norline	65	64	70	10.5 ± 2.6	7.1 ± 1.8
	89	64	76	23.0 ± 4.8	13.0 ± 5.3
	117	67	76	68.0 ± 11.5	22.6 ± 6.0
Coker	65	88	80	6.8 ± 1.8	5.4 ± 1.5
	89	94	82	17.2 ± 4.7	10.8 ± 4.7
	117	94	82	74.0 ± 8.2	25.3 ± 4.0
Windsor	65	72	70	4.6 ± 1.7	4.4 ± 1.2
	89	84	76	12.1 ± 3.4	8.1 ± 2.9
	117	84	76	71.0 ± 10.9	22.1 ± 3.8
Moregrain	65	94	82	8.1 ± 2.8	6.2 ± 2.3
	89	96	94	21.4 ± 5.8	11.5 ± 4.1
	117	96	94	74.9 ± 8.6	23.3 ± 3.6
Roanoke	65	92	74	8.8 ± 2.8	5.9 ± 1.8
	89	94	84	19.3 ± 6.0	11.1 ± 3.6
	117	94	86	65.3 ± 9.2	24.8 ± 5.0

[a]Seeds of *Avena sativa*, cvs. Norline, Windsor, Coker, Moregrain and Roanoke were treated, sown in Petri dishes, incubated and percent germination quantified and root lengths measured as in Table 4 except that the incubation times were 65, 89 and 117 hr; the differences in root lengths between treated and nontreated seeds of every cultivar were statistically significant at all times tested except for cultivar Windsor at 65 hr.

Table 7. Effect of aflatoxin on seed germination and root elongation of various cultivars of *Hordeum vulgare*.[a]

Hordeum vulgare cultivar	Time, hr	Germination, %		Root length, mm	
		No aflatoxin	With aflatoxin	No aflatoxin	With aflatoxin
Surry	39	62	40	5.8 ± 2.1	4.5 ± 1.6
	63	78	56	21.1 ± 5.8	13.1 ± 3.8
	89	78	60	52.4 ± 7.0	21.1 ± 3.7
Barsoy	39	92	90	6.3 ± 4.0	4.0 ± 1.4
	63	92	90	18.9 ± 4.8	10.8 ± 3.0
	89	94	90	42.2 ± 7.8	16.3 ± 3.9
Volbar	39	96	94	7.1 ± 2.5	4.6 ± 1.4
	63	96	100	24.5 ± 3.8	12.8 ± 2.5
	89	96	100	44.4 ± 6.3	18.9 ± 4.1
McNair	39	40	34	5.6 ± 2.0	4.1 ± 1.4
	63	54	42	22.3 ± 7.1	14.1 ± 4.3
	89	56	46	45.1 ± 10.7	17.5 ± 5.8
Henry	39	80	78	6.0 ± 2.1	5.1 ± 2.1
	63	86	86	25.0 ± 7.2	16.0 ± 3.2
	89	90	86	51.6 ± 6.5	19.5 ± 4.7

[a]Seeds of *Hordeum vulgare*, cv. Surry, Barsoy, Volbar, McNair and Henry were treated, sown in Petri dishes, incubated and percent germination quantified and root lengths measured as in Table 4 except that 10 seeds and 10 ml of test solution were added per dish and the imbibition times were 39, 63 and 89 hr; the differences in root lengths between treated and non-treated seeds of every cultivar were statistically significant at all times tested with the exception of cultivar Henry at 39 hr.

during the years 1968-1975, 3 of 416 oat and 0 of 254 barley samples examined contained AFB_1 (*7*).

Are Pollen and Seed Germinations Equally Sensitive to Aflatoxins?

Because both the types of the exogenously supplied aflatoxins and probably their uptakes are variable for the plant systems summarized in Table 8, it may be somewhat misleading to compare the effects of aflatoxins on percent germination of various seeds with those of pollen. However, when such a comparison is made, it is apparent that concentrations above 30-50 μg/ml are usually required to impair imbibition in a solution containing 5.8 and 11.6 μg/ml AFB_1 for 18 hr resulted in 40 and 80% germination except for *Glycine max*. In the latter, inhibitions of seed germination. In contrast, when the medium was supplemented with 3.0*mM* KH_2PO_4, administration of 25 and 30 μg/ml AFB_1 for 4 hr reduced pollen germination by 27.3 and 45.1%. However, mixed aflatoxins consisting primarily of AFG_1 do not inhibit germination either with or without supplementing the medium with KH_2PO_4. This suggests that one reason for the failure of investigators to observe an effect of aflatoxin on seed germination at rather high aflatoxin concentrations could have resulted from the use of mixed aflatoxins rather than pure AFB_1.

Table 8. Summary of the effects of aflatoxins on seed germination.

Plant	Effect	Investigator(s)
Lepidium sativum	No impairment at 1, 2, 5, and 10 μg/ml; 35, 90, and 100% inhibition at 25, 50 and 100 μg/ml, respectively	Schoental and White (*17*)
Variety of seeds	Variable % inhibition (as high as 100 in *Phalaris canariensis*) at 20 μg/ml	Jacquet et al. (*18*)
Lactuca sativa 30 cultivars	No inhibition as high as 1,000 μg/ml in one cultivaor nor by 100 μg/ml in the 29 other cultivars	Crisan (*19*)
Species of *Cruciferae* (19 plants belonging to 11 species)	No effect at 100 μg/ml	Crisan (*20*)
Vigna sinensis	100% inhibition above 50 μg/ml	Adekunle and Bassir (*21*)
Glycine max, cv. Essex	Inhibitions were 5, 20, 40 and 80 or 6, 4, 13 and 19% for seeds exposed 18 and 36 hr respectively to 0.38, 2.90, 5.80 and 11.60 μg/ml AFB_1	Jones et al (*10*)
Onoclea sensibilis	Inhibition of spore germinations was 6.7, 7.8, 27.0, 32.6, and 43.8% at 0.78, 1.56, 2.34, 3.13 and 3.90 μg/ml AFB_1; this same concentration series yielded mean protonemal cells of 2.55, 2.4, 1.5, 1.3 and 1.4 and 3.0 for the control	Cahill et al. (*22*)
Arachis hypogaea	No significant effect of 31.5 μg/ml mixed aflatoxins at 72, 144, 168 and 240 hr	Dashek et al. (present work)
Avena sativa	No significant effect of 31.5 μg/ml mixed aflatoxins at 65, 89 and 117 hr for cvs. Norline, Windsor or Moregrain; 20% inhibition for cvs. Coker and Roanoke at 89 and 117 hr	Dashek et al. (present work)
Hordeum vulgare	No significant effect of 31.5 μg/ml mixed aflatoxins at 39, 63 and 89 hr for cvs. Barsoy, Volbar and Henry; 17-23% inhibitions for Surry and McNair at 39, 63 and 89 hr, respectively	Dashek et al. (present work)
Zea mays	No effect at 0.36, 0.73, 1.5 and 2.9 μg/ml; 23% reduction at 5.8 and 11.6 μg/ml	Dashek et al. (present work)
Lilium longiflorum, cv. Ace pollen	No inhibitory effect from 5-30 μg/ml AFB_1 without 3.0 mM KH_2PO_4; 10.6, 27.3 and 45.1% inhibition at 15, 25 and 30 μg/ml plus 3.00*mM* KH_2PO_4	Jones et al (*10*); Dashek et at (present work)
	No inhibitory effect at 16.64 or 33.28 (5 μg/ml B_1, 0.2 μg/ml B_2, 27.5 μg/ml G_1, 0.58 μg/ml G_2) mixed aflatoxins	

Is Pollen Tube Elongation More or Less Sensitive to Aflatoxins Than the Elongations of Tissues on a Variety of Plants?

Table 9 demonstrates that aflatoxin concentrations > 30 μg/ml are required to inhibit the elongation of tissues of the majority of those plants thus far examined. This comparison may be more meaningful than that for germination since AFB_1 was the aflatoxin of choice in most of the investigations summarized in Table 9. However, data on toxin uptake for the various systems are not available. Given this limitation, it appears that the elongation of *Lilium longiflorum*, cv. Ace pollen tubes is no more sensitive to aflatoxins than that of a variety of plant tissues. Furthermore, tube elongation is considerably less responsive to AFB_1 than *Onoclea sensibilis* protonemal development and *Glycine max* root elongation.

Is the Pollen Bioassay as Sensitive to Aflatoxins as the Commonly Employed Animal Bioassays?

Comparison of the summaries for the effects of aflatoxins on germination (Table 8) and elongation (Table 9) of lily pollen with the effects on various animal systems (Table 10) reveals that pollen bioassay is less sensitive than the commonly employed animal bioassays.

Table 9. Summary of the effects of aflatoxins on growth and elongation of various plants.

Plant	Effect	Investigator(s)
Caralluma frerei	30 ppm similar to nontreated, death and prevention of growth of upper leaves and floral buds at 100 and 300 ppm	Reiss (*23*)
Phalaris canariensis	Radicle elongation impaired by as much as 100% at 50 μg/ml mixture of aflatoxins	Jacquet et al. (*18*)
Lepidium sativum	No inhibition of hypocotyl elongation at 1 μg/ml AFB_1, 14.2 and 58.0% inhibition at 10 and 100 μg/ml; no inhibition of radicle elongation at 1 μg/ml AFB_1; 23.9% inhibitions at 10 and 100 μg/ml	Reiss (*24*)
Chlorella pyrenoidosa	Inhibition of growth of four strains by AFB_1 at 1 μg/ml	Sullivan and Ikawa (*25*)
Glycine max, cv. Essex	Nontreated excised roots followed a sigmoidal growth curve with a dry wt. increase from 100% (0 hr) to 108.5% (24 hr); 4.5% dry wt. decline at 4 hr increase to 101.5% (8 hr); and decrease to 99% (12 hr) at 20 μg/ml AFB_1	Young et al. (*26*)
Kalanchoe diagremontiana	Inhibition of root elongation by approximately 50% at 100 μg/ml AFB_1	Reiss (*27*)
Glycine max, cv. Essex	% inhibition of attached root elongation was 14 (48 hr) and 26 (140 hr) at 2.9 μg/ml AFB_1; 21 (48 hr) and 35 (140 hr) at 5.8 μg/ml; and 36 (48 hr) and 50 (140 hr) at 11.6 μg/ml	Jones et al (*10*)
Arachis hypogaea	No significant effect of mixed aflatoxin at 31.5 μg/ml on root elongation at 72, 144, 168 and 240 hr	Dashek et al. (present work)
Avena sativa five cultivars	Percent inhibition of root elongation at 31.5 μg/ml mixed aflatoxins ranged from 4.3 to 68.8% for 5 cvs. imbibed 65 and 117 hr. respectively	Dashek et al. (present work)
Hordeum vulgare	Percent inhibition of root elongation at 31.5 μg/ml mixed aflatoxins ranged from 22.4 to 62.2 for 5 seeds imbibed 39 and 89 hr. respectively	Dashek et al. (present work)
Zea mays	Mixed aflatoxins at 0.36, 1.45, 2.90 and 5.80 μg/ml stimulated root elongation by 6.1, 30.6, 38.8 and 12.2%	Dashek et al. (present work)
Lilium longiflorum	Maximum inhibition of elongation occurred at 25 (23%) and 30 (36%) μg/ml AFB_1 when 3.0*mM* KH_2PO_4 was added to the germination medium	Jones et al. (*10*)
	Elongation inhibited 18.9% and 23.7% by 16.64 and 33.28 μg/ml mixed aflatoxins	

Table 10. Animal bioassays for aflatoxins B_1.

Concentration	System	Response	Investigator(s)
0.5 μg/ml AFB_1, 24 hr	*Artemia salina*	90% mortality	Abedi and McKinley (*28*)
1.0 μg/ml AFB_1, 24 hr		61% "	
0.048 μg/ml AFB_1, air cell injection 21 days	Chicken embryo	LD_{50}	Verrett et al. (*29*)
0.025 μg/ml AFB_1, yolk injection		"	
18.2 μg AFB_1/50g ducklings	Ducklings	Oral 7 day LD_{50}	Kraybill as cited in Brown (*1*)
16.6 μg AFM_1/50g ducklings		"	
39.2 μg AFG_1/50g ducklings		"	
62.0 μg AFM_2/50g ducklings		"	
84.4 μg AFB_2/50g ducklings		"	
17.2 μg AFB_2/50g ducklings		"	
0.05 to 40 μg AFB_1/ml for 3-5 hr	*Bankia setaceae*	Treated-single cell containing many nuclei, control-two or more cells	Townsley and Lee (*30*)
1 μg/ml for 30 min	*Brachydanio rerio*	Abnormal movement of larvae within 30 min; moribund in 5 to 6 hr; yolk sphere darkening within 20 hr; larval death 24 to 36 hr	Abedi and McKinley (*28*)
1 ppb AFB_1 in diet	Shasta strain rainbow trout	Liver cancer	Sinnhuber et al. (*31*)
8-20 ppb AFB_1 in diet		Visible hepatomas, 4-6 months	
Immersion of embryo for 60 min in AFB_1 at 0.5 μg/ml		High incidence of liver cancer some months later	
4 ppb AFB_1	*Salmo gairdneri*	25 and 48% hepatocellular carcinoma incidence at 9 and 12 months	Masri et al. (*32*)
20 ppb AFB_1		56 and 83% incidences at 8 and 12 months	

Is the Pollen Bioassay as Sensitive as the Available Analytical Methods for Quantitating Aflatoxins?

Comparison of the data in Tables 8 and 9 with those in Table 11 shows that the pollen aflatoxin bioassay is not as sensitive as the current analytical methodology for quantifying aflatoxins.

Does the Pollen Bioassay System Have Any Utilitarian Value?

The value of this system is in its rapidity and inexpensiveness as well as the fact that one does not need sophisticated instrumentation and a highly trained technician to perform the bioassay. Furthermore, pollen germination and subsequent tube elongation are especially sensitive to AFG_1. However, these advantages are offset by the lack of sensitivity of the bioassay. We suggest that *Onoclea sensibilis* spores and/or *Glycine max* seeds be adopted as organisms of choice for the development of those aflatoxin bioassays which would employ plants.

Is the Pollen Bioassay as Sensitive to Aflatoxins as the Commonly Employed Animal Bioassays?

Comparison of the summaries for the effects of aflatoxins on germination (Table 8) and elongation (Table 9) of lily pollen with the effects on various animal systems (Table 10) reveals that the pollen bioassay is less sensitive than the commonly employed animal bioassays.

Table 11. Comparison of sensitivities of analytical methodology for quantifying aflatoxins.

Technique	Sensitivity	Error	Reference
Thin layer chromatography			
Plus visual identification of B_1, B_2, G_1 and G_2 by fluorescence intensity	0.3-0.4 ng	20-28%	Coomes et al (*33*)
plus fluorodensitometry	0.25-1.5 × 10^3 μg	?	Ayres and Sinnhuber (*34*)
Plus silica gel minicolumn cleanup and identification of B_1 and M_1 with TFA	0.05 μg/kg	Recoveries B_1 85% M_1 65%	Van Egmond (*35*)
Plus silica gel cleanup with addition of citric acid to extracting solvent and ammonium sulfate to the extract solution; methanol substituted for acetone during elution from silica gel	0.1-0.2 ng, B_1 and M_1 added	Recoveries 81 ± 6 B_1 (0.1 μg added) 91 ± 17 B_1 (0.2 μg added) 66 ± 12 M_1 (0.1 μg added) 82 ± 19 M_1 (0.2 μg added)	Truckess and Stoloff (*36*)
Plus laser fluorometric determination	10-1000 pg	rMS% error 26	Diebold et al. (*37*)
High pressure liquid chromatography			
Waters Associates Model 440 absorbance detector	10 ng for B_1 and B_2	Recovery of B_1 and B_2 90-95%	Pons and Franz (*38*)
Shimadzu Model RF-510LC spectroflurometer	Relationship between fluorescence peak area and the amount injected linear in the range of 0.3 ng to 120 ng	?	Manabe et al. (*39*)
Fluorescence detection with a packed silica gel flow cell	0.1 ng	?	Blanc (*40*)
Oscillopolarographic traces	0.3- 50 μg for B_1 and G_1	?	Garjan et al. (*41*)
Ultraviolet light absorption spectrophotometry for B_1	3-10 μg		Nabney and Nesbitt (*42*)
Sephadex gel filtration G-10 elution with 1% aqueous method	10 μg	10%	Manabe et al. (*43*)

Is the Pollen Bioassay as Sensitive as the Available Analytical Methods for Quantitating Aflatoxins?

Comparison of the data in Tables 8 and 9 with those in Table 11 shows that the pollen aflatoxin bioassay is not as sensitive as the current analytical methodology for quantifying aflatoxins.

Does the Pollen Bioassay System have any Utilitarian Value?

Experimental data indicate that pollen systems are not a particularly useful bioassay for aflatoxin hepatocarcinogenicity.

We thank Mr. John Kinzie for preparing mixed aflatoxins and Mr. Thomas Eadie, Mr. Paul B. Ferrara and Mr. H. Franklin McGowan of the Virginia Division of General Services, Bureau of Product Regulation for aflatoxin analyses. Appreciation is extended to The Seed Testing Laboratory of the Virginia Department of Agriculture and Consumer Services for providing seeds and their cooperation in this investigation. We are grateful to Ms. Pat Drake for typing.

These studies were aided by Grant IN-127 from the American Cancer Society, funds from the West Virginia University Department of Biology and travel funds from the National Institute of Environmental Health Sciences and the Environmental Protection Agency (to W. V. D.) and funds from the Department of Biology, Virginia Commonwealth University, Richmond, Va. (to G. C. L.).

REFERENCES

1. Brown, R. F. Some bioassay methods for mycotoxins. In: Proc. First US-Japan Conference on Toxic Micro-Organisms, Mycotoxins. Botulism, M. Herzberg, Ed., UJNR Joint Panels on Toxic Micro-Organisms and the U.S. Department of the Interior, 1970, p. 12.
2. Dashek, W. V., and Llewellyn, G. C. Aflatoxin toxicity and mode of action in plant tissues. Ann. Nutr. Alim. 31: 841 (1977).
3. Dashek, W. V., and Llewellyn, G. C. The influence of the carcinogen aflatoxin B_1 on the metabolism of germinating lily pollen. In: Fertilization in Higher Plants, H. F. Linskens,

Ed., North Holland, Amsterdam, 1974, p. 351.
4. Dickinson, D. B. Germination of lily pollen: respiration and tube growth. Science 150: 1818 (1965).
5. Horowitz, W., Senzel, A., Reynolds, H., and Parker, D. L. Official Method of Analysis of the Association of Official Analytical Chemists, Section 26.019. Washington, D.C., 1975, p. 466.
6. Llewellyn, G. C., Carlton, W. W., Robbers, J. E., and Hansen, W. G. A rapid and simplified method for the preparation of pure aflatoxin B_1. In: Developments in Industrial Microbiology, Vol. 14, 1973, p. 325.
7. Stoloff, L. Aflatoxins—an overview. In: Mycotoxins in Human and Animal Health, J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman, Eds. Pathotox Publishers, Park Forest South, Ill., 1977, p. 7.
8. Dashek, W. V., Jones, H. C., Chancey, J. C., Morton, W. A., and Llewellyn, G. C. Toxic responses of germinating soya-beans and pollen to aflatoxins. Toxicon 17: 34 (1979).
9. Danley, J. M., Jones, H. C., Llewellyn, G. C., and Dashek, W. V. Toward an understanding of the mode of action and metabolism of aflatoxins in soybeans. In: Proceedings of 4th IUPAC Symposium on Mycotoxins and Phycotoxins, P. Krogh, Ed., Pathotox Publishers, Park Forest South, Ill., in press.
10. Jones, H. C., Chancey, J. C., Morton, W. A., and Llewellyn, G. C. Toxic responses of germinating pollen and soybeans to aflatoxins. Mycopathologia, in press.
11. Mislivec, P. B., and Bruce, W. R. Incidence of toxic and other mold species and genera in soybeans. J. Food Protection 40: 309 (1977).
12. Shotwell, O. L., Hesseltine, C. W., Burmeister, H. R., Kwolek, W. F., Shannon, G. M., and Hall, H. H. Survey of cereal grains and soybeans for the presence of aflatoxin. II. Corn and soybeans. Cereal Chem. 46: 454 (1969).
13. Shotwell, O. L., Hesseltine, C. W., and Gouden, M. L. Incidence of aflatoxin in southern corn, 1969-1970. Cereal Sci. Today 18: 192 (1973).
14. Shotwell, O. L., Kwolek, W. F., Goulden, M. L., Jackson, L. K., and Hesseltine, C. W. Aflatoxin occurrence in some white corn under loan. I. Incidence and level. Cereal Chem. 52: 373 (1975).
15. McMillan, W. W., Wilson, D. M., and Widstrom, N. W. Insect damage, *Aspergillus flavus* ear mold, and aflatoxin contamination in South Georgia corn fields in 1977. J. Environ. Qual. 7: 564 (1978).
16. Wilson, D. M., McMillan, W. W., and Widstrom, N. W. Field contamination of corn in South Georgia. J. Assoc. Off. Anal. Chemists 56: 798 (1979).
17. Schoental, R., and White, A. F. Aflatoxin and "albinism" in plants. Nature 205: 57 (1965).
18. Jacquet, J., Boutibonnes, P., and Saint, S. Biological effects of the aflatoxins of *Aspergillus parasiticus*. Rev. Immunol. 35: 159 (1971).
19. Crisan, E. V. Effects of aflatoxin on germination and growth of lettuce. Appl. Microbiol. 25: 342 (1973).
20. Crisan, E. V. Effects of aflatoxin on seedling growth and ultra-structure in plants. Appl. Microbiol. 12: 991 (1973).
21. Adekunle, A. A., and Bassir, O. The effects of aflatoxin B_1 and palmotoxins B_0 and G_0 on the germination and leaf colour of the cowpea (*Vigna sinensis*). Mycopathol. Mycol. Appl. 51: 299 (1973).
22. Cahill, S. G., Fisher, R. W., and Llewellyn, G. C. Examination and evaluation of germination and protonemal development for *Onoclea sensibilis* fern spores treated with aflatoxin B_1. Mycopathologia 64: 43 (1978).
23. Reiss, J. Hemmung des Sprosswachstums von *Caralluma frerei* Rowl. durch Aflatoxin. Planta: 89: 369 (1969).
24. Reiss, J. Hemmung der Keimung der Kresse (*Lepidium sativum*) durch Aflatoxin B_1 and Rubratoxin B_1. Biochem. Physiol. Pflanz. 162: 361 (1971).
25. Sullivan, J. D., Jr., and Ikawa, M. Variations in inhibition of growth of five *Chlorella strains* by mycotoxins and other toxic substances. J. Agr. Food Chem. 20: 921 (1972).
26. Young, J. W., Dashek, W. V., and Llewellyn, G. C. Aflatoxin B_1 influence on excised soya-bean root growth, ^{14}C-leucine uptake and incorporation. Mycophatologia 66: 91 (1978).
27. Reiss, J. Effects of mycotoxins on the development of epiphyllous buds of *Kalanchoe daigremontiana*. Z. Pflazenphysiol. 82: 446 (1977).
28. Abedi, Z. H., and McKinley, W. P. Zebra fish eggs and larvae as aflatoxin bioassay test organisms. J. Assoc. Offic. Anal. Chemists 51: 902 (1968).
29. Verrett, M. J., Marliac, J. P., and McLaughlin, J. Use of the chick embryo in the assay of aflatoxin toxicity. J. Assoc. Offic. Anal. Chemists 47: 1003 (1964).
30. Townsley, P. M., and Lee, E. G. H. Response of fertilized eggs of the mollusc, *Bankia setaceae*, to aflatoxin. J. Assoc. Offic. Anal. Chemists 50: 361 (1967).
31. Sinnhuber, R. O., Walls, J. H., Hendricks, J. D., Putman, G. B., Nixon, J. E., and Pawlowski, N. E. Trout bioassay of mycotoxins. In: Mycotoxins in Human and Animal Health, J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, Eds., Pathotox Publishers, Park Forest South, Ill. 1977, pp. 731-744.
32. Masri, M. S., Hendricks, J. D., and Sinnhuber, R. O. Aflatoxin Q_1—Major detoxifying pathway in primates, carcinogenicity tests in rainbow trout. Toxicon 17: 116 (1979).
33. Coomes, T. J., Crowther, P. C., Francis, B. J., and Stevens, L. The detection and estimation of aflatoxins in groundnuts and groundnut materials. Analyst 90: 492 (1965).
34. Ayres, J. L., and Sinnhuber, R. O. Flurodensitometry of aflatoxin on thin-layer plates. J. Am Oil Chemists. Soc. 43: 423 (1966).
35. Van Egmond, H. P. Two-dimensional chromatographic method for determination of aflatoxins in liver. Occurrence of aflatoxins in animal liver. In: Proceedings 4th IUPAC Symposium on Mycotoxins and Phycotoxins, P. Krogh, Ed., Pathotox Press, Park Forest South, Ill., in press.
36. Truckess, M. W., and Stoloff, L. Extraction, cleanup and quantitative determination of aflatoxins B_1 and M_1 in beef liver. J. Assoc. Off. Anal. Chemists 62: 1080 (1979).
37. Diebold, G. J., Karny, N., Zare, R. N., and Seitz, L. M. Mycotoxins: Laser flurometric determination of aflatoxin B_1 in corn. J. Assoc. Off. Anal. Chemists 62: 564 (1979).
38. Pons, W. A., Jr., and Franz, A. O., Jr., High performance liquid chromatography of aflatoxins in cottonseed products. J. Assoc. Off. Anal. Chemists 60: 89 (1977).
39. Manabe, M., Goto, T., and Matsuura, S. 1978. High-performance liquid chromatography of aflatoxins with fluorescence detection. Agr. Biol. Chem. 42: 2003 (1978).
40. Blanc, M. Use of high-performance liquid chromatography combined with fluorescence detection using a packed silicagel flow cell for the quantity analysis of aflatoxins B_1 and M_1 in foods. In: Proc. 4th IUPAC Symposium on Mycotoxins and Phycotoxins, P. Krogh, Ed., Pathotox Press, Park Forest South, Ill., in press.
41. Garjan, R. J., Nesheim, S., and Campbell, A. D. Note on identification of aflatoxins by oscillographic polarography. J. Assoc. Offic. Agr. Chemists 47: 27 (1964).
42. Nabney, J., and Nesbitt, B. F. A spectrophotometric method for determining the aflatoxins. Analyst 90: 155 (1965).
43. Manabe, M., Matsuura, S., and Nakano, M. Isolation and quantitative analysis of four aflatoxins (B_1, B_2, G_1 and G_2) by

thin-layer and liquid chromatography. In: Proceedings First US-Japan Conference on Toxic Micro-Organisms, Mycotoxins, Botulism. H. Herzberg, Ed., UJNR Joint Panels on Toxic Micro-Organisms and the U.S. Department of the Interior, 1970, p. 23.